SCIENTIFIC ABSTRACT

The goal of this project is to test the use of "suicidal lymphocytes" as a means of controlling graft-versus-host disease (GVHD) thereby expanding the population of patients able to benefit from the graft-vs.-leukemia effect (GVL) associated with allogeneic transplantation. The ability to fully harness the immunologically mediated graft-versus-leukemia (GVL) effect noted after allogeneic marrow or stem cell transplantation is limited by the development of GVHD. Current therapies, designed to prevent or treat GVHD are sub optimal and the risk of GVHD remains a major barrier preventing many leukemia patients from undergoing allogeneic transplantation and benefiting from a GVL effect. A novel strategy to control GVHD is to **selectively** eliminate the GVHD initiating T-cell after infusion, instead of suppressing the function of all T-cells. This selectivity is generated by transducing T-cells *ex-vivo* with a retrovirus containing the herpes simplex virus-thymidine kinase (HSV-*TK*) gene. These "suicidal" lymphocytes are then infused into the patient. Should GVHD develop the "suicidal lymphocytes" are eliminated by the administration of ganciclovir (GCV) to which they are now sensitive.

Several small clinical trials have demonstrated proof of principle but have also highlighted technical problems that suggest that the production process is adversely affecting the alloreactivity of the suicidal lymphocytes. We have identified several features of the production process that might contribute to this diminished T-cell function and have substantially modified the retroviral vector and the production process in order to produce more functional HSV-TK+ T-cells. We propose to conduct a second generation "suicidal" lymphocyte clinical trial to test whether the promise of this strategy can be realized. If successful then this strategy might remove the risk of GVHD as a barrier to transplantation and enable a greater percentage of leukemia patients to benefit from the GVL effect.

1. **Specific Aim 1: Optimize the production process for the generation of clinical grade suicidal lymphocytes at a clinical scale:** The preclinical work performed to date has used non-GMP grade vector in non-GMP laboratories. The first aim is to verify that we can produce clinical scale numbers of suicidal lymphocytes that meet our potency and release criteria in the setting of a GMP facility using the GMP grade vector. Specifically, variables to be re-validated are: cell concentration, culture vessel, cell culture media, method of stimulation, use of clinically usable transduction facilitators (Fibronectin or protamine sulfate in place of polybrene) the transduced cell selection process (ClinicMACS instead of AutoMACS). Additionally, quality measures including: measures of suicidal lymphocyte subset composition (CD4, CD8, CD56 etc), diversity (TCR-Vβ repertoire) and immune function (Chromium release assay, CD4 & CD8 activity after CMV stimulation using cytokine flow staining after HLA-A2 peptide tetramer or ectopic peptide stimulation) need to be re-tested in the GMP production setting. The method yielding the highest number of suicidal lymphocytes with the best alloreactivity will be chosen for use in the clinical trial.

2. **Specific Aim 2: Conduct a clinical trial evaluating the use of suicidal lymphocytes:** High risk patients with CML, AML or MDS will receive a T-cell depleted allogeneic stem cell supplemented with 5 x 10^7 suicidal lymphocytes after receiving a preparative regimen of intravenous Busulfan and Fludarbine. Cohorts will receive GVHD prophylaxis of decreasing duration. Objectives of the clinical trial are:
    a. To determine if GVHD can be controlled in patients receiving allogeneic transplantation with CD34+ stem cells and HSV-TK+ T-lymphocytes (suicidal lymphocytes).

b. To determine if immunosuppressive GVHD prophylaxis using tacrolimus is required in conjunction with the use of suici dal lymphocytes.
c. To assess whether use of suicidal lymphocytes has any adverse effect on engraftment of donor hematopoietic cells.
d. To assess disease response and survival.